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## Certificate of Analysis

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## CviJ I

Catalog No: 2125

**Lot No:** See Product Label

**Package Size:** See Product Label

**Concentration:** See Product Label

**Storage Conditions:** Store at -80°C

**Note:** Mix well after thawing. Avoid multiple freeze/thaw cycles.

### Source

Purified from E coli strain that carries the cloned cvjRI gene from *Chlorella virus IL-3A* (US Patent No. US005472872A)

### Recognition/Cut Site

5'-PuG↓CPy-3'  
3'-PyC↑GPy-5'

### Applications

- CviJ I is the only available restriction endonuclease recognizing two-three base pair sequence (1,2,3), thus allowing for new research applications (4).
- This recombinant version of CviJ I cleaves only PuGCPy sites (4).
- Suitable for high resolution mapping of short DNAs like amplified products or small plasmids.
- Other CviJ I applications, like shotgun cloning, thermal cycle labeling (5) or epitope mapping can be performed either with this version or with CviJ I.\*

### Unit Definition

One unit is the amount of enzyme required to completely digest 1 µg of pBR322 DNA in 1 hour at 37°C in a total reaction volume of 25 µl.

### Heat Inactivation

10 minutes at 65°C

### Reaction Temperature

37°C

### Assay Conditions

20 mM glycyglycine-KOH (pH 8.5)  
10 mM Mg Acetate  
0.1 mM DTT  
50 mM K Acetate  
10% DMSO  
1 µg of pBR322 DNA  
Reaction volume of 25 µl

### Storage Buffer

20 mM Tris-Acetate (pH 8.0)  
0.5 mM EDTA  
0.1 mM dithiothreitol  
50 mM K Acetate  
5 mM MgCl<sub>2</sub>  
50% (v/v) glycerol

### Quality Control

**Endonuclease:** Incubation of 2 units of CviJ I with 0.25 µg of pBR322 DNA at 37°C for 16 hours (a 128-fold over-digestion) resulted in the same sharp characteristic banding pattern observed with 2 units of CviJ I and 0.25 µg of pBR322 DNA in the standard 1 hour assay reaction, as determined by agarose gel electrophoresis. Reaction volume of 50 µl.

**3'-Exonuclease:** Incubation of 0.5, 1.0, and 2.0 units of CviJ I with 5 pmoles of lambda/Taq I fragments (3'-labeled with Klenow exo- and [<sup>3</sup>H]dCTP), incubated for 1 hour at 37°C resulted in ≤5% slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

**5'-Exonuclease/5'-Phosphatase:** Incubation of 0.5, 1.0, and 2.0 units of CviJ I with 0.25 µg of [5'-<sup>32</sup>P]lambda/Hae III fragments for 1 hour at 37°C resulted in ≤5% slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

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### References

1. Xia, Y., Burbank, D., Uher, L., Rabussay, D. and Van Etten, J. *Nucleic Acids Res.* 15, 6075-6090
2. Fitzgerald, M.C., Skowron, P., Van Etten, J.L., Smith, L.M. and Mead, D.A. (1992) *Nucleic Acids Res.* 20, 3753-3762
3. Mead, D., Swaminathan, N., Van Etten J. and Skowron, P.M. *Recombinant CviJI restriction endonuclease.* (1995) *Unites States Patent no US005472872A*
4. Skowron, P.M, Swaminathan, N., McMaster, K., George, D., Van Etten, J. and Mead, D. *Gene* 157 (1995) 37-41
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